Search Pgs 1 3

WEST Search History

DATE: Tuesday, February 11, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L1	crystal$ near3 ige	5	L1

END OF SEARCH HISTORY

WEST

Generate Collection | Print

Search Results - Record(s) 1 through 5 of 5 returned.

☐ 1. Document ID: US 20030003502 A1

L1: Entry 1 of 5 File: PGPB Jan 2, 2003

PGPUB-DOCUMENT-NUMBER: 20030003502
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030003502 A1

TITLE: Three-dimensional model of a complex between a Fc epsilon receptor alpha chain and a Fc region of an IgE antibody and uses thereof

PUBLICATION-DATE: January 2, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Jardetzky, Theodore S.	Chicago	IL	US	
Garman, Scott Clayton	Rockville	MD	US	
Wurzburg, Beth A.	Evanston	IL	US	
Kinet, Jean-Pierre	Lexington	MA	US	

US-CL-CURRENT: 435/7.1; 702/19, 703/11

ABSTRACT:

The present invention includes three-dimensional models of complexes between antibody receptor proteins, such as Fc.epsilon.RI.alpha. proteins, and antibodies, such as Fc-C.epsilon.3/C.epsilon.4 regions of IgE antibodies, as well as methods to produce such models. The present invention also includes muteins having increased stability and/or antibody binding activity, as well as methods to produce such muteins, preferably using information derived from three-dimensional models of the present invention. Also included are nucleic acid sequences encoding muteins of the present invention and use of those sequences to produce such muteins. Also included is the use of the model to identify compounds that inhibit the binding of an antibody receptor protein to an antibody. The present invention also includes uses of such muteins and inhibitory compounds, for example, in methods to diagnose and protect animals from allergy and other abnormal immune responses.

L1: Entry 1 of 5 File: PGPB Jan 2, 2003

DOCUMENT-IDENTIFIER: US 20030003502 A1
TITLE: Three-dimensional model of a complex between a Fc epsilon receptor alpha chain and a Fc region of an IgE antibody and uses thereof

Detail Description Paragraph (19):
[0041] A model of the present invention also provides additional information that is not available from other sources. For example, a model can identify the crystal contacts between crystals and predict the location of the IgE binding domain, including those amino acids that actually form contacts with a Fc domain of an IgE antibody, such as those in the binding face of the Fc.epsilon.RI.alpha. protein. A model can also identify the amino acids in the interface between domain 1 and domain 2 (i.e., the D1D2 interface), as well as those in the cleft formed between the two domains of the Fc.epsilon.RI.alpha. protein. Particularly important regions of the

complex indicated by the model represented in Table 1 include, but are not limited to, Fc.epsilon.RI.alpha.:Fc-C.epsilo- n.3/C.epsilon.4 interaction site 1, Fc.epsilon.RI.alpha.:Fc-C.epsilon.3/C.- epsilon.4 interaction site 2, the hinge between domain C.epsilon.3 and domain C.epsilon.4 of the Fc-C.epsilon.3/C.epsilon.4 region, and a FcERIa:Fc-C.epsilon.3/C.epsilon.4 region that interacts with 3-[3-(cholamidopropyl) dimethylammonio]-1-propane-sulfonate (CHAPS). Interaction sites 1 and 2 are the sites at which amino acids from Fc.epsilon.RI.alpha. and Fc-C.epsilon.3/C.epsilon.4 interact with each other. These sites are described in more detail in the Examples and represent sites to target for drug design and mutein production.

Detail Description Paragraph (94):
[0115] The initiation of IgE-mediated allergic responses requires the binding of IgE antibody to its high affinity receptor, Fc.epsilon.RI. Crosslinking of Fc.epsilon.RI initiates an intracellular signal transduction cascade that triggers the release of mediators of the allergic response. The interaction of IgE-Fc domains with Fc.epsilon.RI is a key recognition event that is central to this process and mediated by the extracellular domains of the .alpha.-chain of Fc.epsilon.RI. This Example describes the solution of a crystal structure of the human IgE-Fc:Fc.epsilon.RI.alpha. complex, the coordinates of which are disclosed in Table 1. The crystal structure reveals that one receptor binds one IgE-Fc asymmetrically through interactions at two sites involving both N-terminal IgE-Fc C.epsilon.3 domains. The interaction of one receptor with IgE-Fc blocks the high-affinity binding of a second receptor and features of this interaction are conserved in other Fc receptor family members. The structural analysis suggests new approaches to the inhibition of IgE binding to Fc.epsilon.RI for the treatment of allergy and asthma.

Detail Description Paragraph (99):
[0120] These questions are addressed with the solution of a crystal structure of a complex of the human IgE-Fc with Fc.epsilon.RI as disclosed herein as well as of a crystal structure of the unbound IgE-Fc fragment as disclosed in 60/189,403, ibid. The structure of the complex reveals two interaction sites for the IgE-Fc on the receptor surface and clarifies how a 1:1 complex between antibody and receptor is formed. The two IgE-Fc C.epsilon.3 domains bind to distinct sites on the receptor; one is formed by the C-C' loop in the receptor D2 domain, while the second site involves the four solvent-exposed tryptophans. The IgE C.epsilon.4 domains do not form direct contacts with the receptor and point away from the C.epsilon.3 interaction sites. The structure of the complex accounts for previous mutagenesis and structural observations and shows that the Fc forms a complementary crown across the convex surface of the receptor. Comparison of the complex with the isolated IgE-Fc crystal structure suggests that large structural changes may occur upon IgE binding to its receptor (see No. 60/189,403, ibid.) The IgE-Fc:Fc.epsilon.RI.alpha. complex provides a model for understanding the function of other antibody Fc-receptors and new conceptual approaches to the inhibition of IgE-mediated diseases.

Detail Description Paragraph (101):
[0122] The crystallization of the IgE-Fc:Fc.epsilon.RI.alpha. complex required the expression of each protein using recombinant baculovirus technology. The expression of the Fc.epsilon.RI.alpha. was carried out essentially as described previously.sup.11. The IgE heavy chain contains four constant domains (C.epsilon.1-C.epsilon.4), in contrast to the three found in IgG antibodies. The interaction of Fc.epsilon.RI with IgE has been previously mapped to the two C-terminal constant domains of the IgE-Fc (domains C.epsilon.3/C.epsilon.4).sup.12-16. The expression and purification of the human IgE-Fc C.epsilon.3/C.epsilon.4 domains was established as described (No. 60/189,403, ibid.) and purified protein used to form complexes with Fc.epsilon.RI.alpha.. The best complex crystals (spacegroup P42.sub.12) obtained with the wild type (wt) Fc.epsilon.RI.alpha. protein were small (.about.60-100 .mu./edge) and diffraction data was limited to a resolution of .about.4.5 .ANG. (Table 3, crystal form I). In order to improve the complex crystals, a triple carbohydrate mutant of Fc.epsilon.RI.alpha. (Fc.epsilon.RI.alpha..DELTA.4- -6) was expressed in insect cells. The Fc.epsilon.RI.alpha..DELTA.4-6 mutant lacks carbohydrate at three of the seven native attachment sites (residues 74, 134, 140) and was previously shown to produce .about.50% of the wt protein in CHO cells.sup.17. Complexes formed with

baculovirus-expressed Fc.epsilon.RI.alpha..alpha.4-6 grow crystals in spacegroup R32 and diffract X-rays to a resolution of 3.25 .ANG. (Table 3, crystal form II). The structure was determined by molecular replacement techniques as described in Methods. Manual model building was done with the program O.sup.18 and refinement carried out with CNS.sup.19. Current refinement statistics for the complex are shown in Table 3, with an overall R-free of 29.3% and R-cryst of 27.0% to 3.25 .ANG.. FIG. 1a shows electron density from a sigmaa-weighted 2Fo-Fc simulated annealing omit map calculated with the current model phases.

Detail Description Paragraph (103):
[0124] Both crystal forms of the IgE-Fc:Fc.epsilon.RI.alpha. complex contain a single 1:1 complex in the asymmetric unit, with similar overall geometric features (FIGS. 1b, c). Given the low resolution of crystal form I, detailed interpretation of the interfaces is limited to crystal form II. Binding interactions are formed exclusively between the N-terminal C.epsilon.3 domains of the IgE-Fc with Fc.epsilon.RI.alpha.. The C.epsilon.4 domains of the IgE-Fc point away from the receptor structure and make no contacts with either receptor domain. The C.epsilon.3/C.epsilon.4 hinge regions are also not involved in direct receptor contacts. The two C.epsilon.3 domains are related by a nearly perfect diad axis (180.7.degree. rotation), except for residues in the C.epsilon.2/C.epsilon.3-linker region (residues 331-336) (FIGS. 1b, c). The C.epsilon.4 domains are also related by a nearly perfect diad axis (179.6.degree. rotation), but the orientation of this axis differs from that determined for the C.epsilon.3 domains (FIGS. 1b,c). The angle between the C.epsilon.3 and C.epsilon.4 domains also differs from that seen in the IgE-Fc alone (see 60/189,403, ibid.) While structured carbohydrate is visible in both the IgE-Fc and Fc.epsilon.RI.alpha. proteins, the carbohydrate groups do not contribute significantly to interactions between the two molecules. In addition, the IgE-Fc carbohydrate does not make any contacts across the IgE-Fc diad axis, but lies along the surface of each IgE-Fc domain.

Detail Description Paragraph (112):
[0133] The IgE-Fc in the complex is observed in a conformation that is very similar to the Fc domains of IgG antibodies.sup.30,31. Similar binding interactions between IgG antibodies and Fc.gamma.Rs could form an analogous 1:1 complex, as suggested by biophysical studies of the IgG-Fc interaction with Fc.gamma.RIII.sup.32. In contrast to the similarities of the bound IgE-Fc to IgG-Fc structures, the crystal structure of the IgE-Fc alone shows a large re-arrangement of the two C.epsilon.3 domains that is greater than the conformational variation observed in IgG-Fc structures (see P_AL-9, ibid.). The IgE-Fc conformation may change substantially from the unbound conformation, which may exist in multiple conformational states that interact weakly with the receptor. This conformational variation in the IgE-Fc structure suggests new avenues to inhibiting IgE-receptor interactions using allosteric modulators that could stabilize the closed, unbound IgE-Fc structure.

Detail Description Paragraph (126):
[0147] The crystal structure of the IgE-Fc:Fc.epsilon.RI.alpha. complex clarifies the atomic interactions that regulate the specificity and stoichiometry of protein:protein interactions underlying allergy and anaphylaxis. Similar complexes may form between IgG antibodes with their receptors, as suggested by previous mutagenesis studies and the structural analysis presented here, in contrast to models proposed for the interaction of IgG-Fc with the low affinity receptor, Fc.gamma.RIIb.sup.25 and Fc.gamma.RIIa.sup.24. Knowledge of these interactions may allow the development of inhibitors for the treatments of allergy and asthma and may also facilitate the targeted engineering of therapeutic antibodies to interact with specific subsets of the FcR family.sup.45.

Detail Description Paragraph (128):
[0149] A model for the formation of a complex between an intact IgG antibody and Fc-receptor is shown in FIG. 6. In this model the crystal structure of the low affinity IgG receptor (Fc.gamma.RIIb).sup.25 and one of the available intact IgG antibody structures (1IGY).sup.46 were superimposed on the IgE-Fc:Fc.epsilon.RI.alpha. complex. Superposition of the IgG structure is based on the Site 2 interactions, and this places the second IgG-Fc Cg2 domain within close proximity of the Site 1 binding surface without any conformational rearrangements (FIG. 6). The Fab arms of IgG are flexible and are also easily accommodated into

this complex. Antigen-induced crosslinking of antibody:FcR complexes, leads to the co-localization of Fc receptors and the initiation of intracellular signal transduction cascades.sup.2,47. Within the one of the IgE-Fc:Fc.epsilon.RI.alpha. crystal forms and the IgE-Fc crystals (60/189,403, ibid.), C.epsilon.3 domains from adjacent molecules are observed to form packing interactions in the crystal through a strand to strand hydrogen-bonding interaction. Such interactions could potentially play a role in orienting crosslinked receptors, allowing the intracellular approach of receptor-associated kinases to adjacent .gamma.-chain cytoplasmic tails, initiating the signal transduction cascade. A potential role for C.epsilon.3:C.epsilon.3 interactions in signal transduction remains to be tested.

Detail Description Paragraph (130):
[0151] 1. Crystallization of the Human IgE-Fc:Fc.epsilon.RI.alpha. Complex

---

☐ 2. Document ID: US 20020058279 A1

L1: Entry 2 of 5 File: PGPB May 16, 2002

PGPUB-DOCUMENT-NUMBER: 20020058279
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020058279 A1

TITLE: Microvolume immunoabsorbant assays with amplified electrochemical detection

PUBLICATION-DATE: May 16, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Fritsch, Ingrid	Fayetteville	AR	US	
Beittle, Robert JR.	Fayetteville	AR	US	

US-CL-CURRENT: 435/6

ABSTRACT:

A structure and method for chemical sensing utilizing microassays. Microcavities or micropores are combined with assay techniques to provide a very fast and very sensitive means of detecting chemical compounds. Assay techniques are modified to include a metal ion binding carrier species especially suitable for use in conjunction with the electrochemical detection. This allows assays to be combined with electrochemical analysis, thus allowing the high speed ease and hypersensitivity available in the invention disclosed herein.

L1: Entry 2 of 5 File: PGPB May 16, 2002

DOCUMENT-IDENTIFIER: US 20020058279 A1
TITLE: Microvolume immunoabsorbant assays with amplified electrochemical detection

Detail Description Paragraph (46):
[0100] Modification and characterization of gold macrosubstrates. Studies using SAMs of MUA and MUOL83-86 for immobilization of protein 83-85 and DNA86 have been previously reported. However, to our knowledge, this is the first report of using MUA and MUOL SAMs for immobilization of rat-anti mouse IgG to gold surfaces in a sandwich-type ELISA for detection of mouse IgG. Consequently, we performed several characterization and activity studies of the modified surfaces. Previously reported studies have used thioctic acid and cysteamine for immobilization of anti human IgE

on piezoelectric quartz crystal with gold electrodes. Thioctic acid SAMs have been used for the detection of mouse IgGI and rabbit IgG.16 Butanethiol SAMs have been used for rabbit IgG detection.16 Photoimmobilization of mouse IgG on Au has been accomplished using SAMs of 10,10'-dithiobis(decanoic acid N-hydroxysuccinimide ester) terminated alkyl disulfide.80 Previous studies have used various SAMs to attach proteins other than IgG.

☐ 3. Document ID: US 20010039479 A1

L1: Entry 3 of 5 File: PGPB Nov 8, 2001

PGPUB-DOCUMENT-NUMBER: 20010039479
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010039479 A1

TITLE: Three-dimensional model of a Fc region of an IgE antibody and uses thereof

PUBLICATION-DATE: November 8, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Jardetzky, Theodore S.	Chicago	IL	US	
Wurzburg, Beth A.	Evanston	IL	US	

US-CL-CURRENT: 702/19; 530/388.1

ABSTRACT:

The present invention includes three-dimensional models of antibodies, such as Fc-C.epsilon.3/C.epsilon.4 regions of IgE antibodies, as well as methods to produce such models. The present invention also includes muteins having increased stability and/or antibody receptor binding activity, as well as methods to produce such muteins, preferably using information derived from three-dimensional models of the present invention. Also included are nucleic acid sequences encoding muteins of the present invention and use of those sequences to produce such muteins. Also included is the use of the model to identify compounds that inhibit the binding of an antibody receptor protein to an antibody. The present invention also includes uses of such muteins and inhibitory compounds, for example, in methods to diagnose and protect animals from allergy and other abnormal immune responses.

L1: Entry 3 of 5 File: PGPB Nov 8, 2001

DOCUMENT-IDENTIFIER: US 20010039479 A1
TITLE: Three-dimensional model of a Fc region of an IgE antibody and uses thereof

Summary of Invention Paragraph (13):
[0012] The present invention also includes an isolated crystal of a human IgE Pc region comprising C.epsilon.3 and C.epsilon.4 domains.

Detail Description Paragraph (82):
[0108] The binding of soluble IgE to its high affinity receptor, Fc.epsilon.RI, is a requisite step in the cascade of events associated with the allergic response and anti-parasitic immunity.sup.1-3. Crosslinking of receptor-bound IgE by antigens triggers intracellular signaling events leading to effector cell activation.sup.4. This example describes the solution of a 2.3 .ANG. crystal structure of the human IgE-Fc domains, C.epsilon.3 and C.epsilon.4, which bind to Fc.epsilon.RI, the

coordinates of which are disclosed in Table 1. The IgE-Fc crystal structure reveals a large (.about.15.degree.) tertiary rearrangement of the C.epsilon.3 domains when compared to IgG-Fc structures and the IgE-Fc bound to Fc.epsilon.RI. The free IgE-Fc adopts a more compact arrangement that places the C.epsilon.3 domains into close proximity in a "closed" configuration, obstructing receptor-binding loops. This IgE-Fc conformational change is mediated by three flexible segments that lie within the C.epsilon.3 domain and not by the interdomain connecting loop. The "closed" structure of the IgE-Fc highlights the potential for novel conformational variation in the effector domains of different antibody classes and suggests new strategies for the design of therapeutic compounds for the treatment of allergy and asthma.

Detail Description Paragraph (84):
[0110] IgE antibodies consist of two Fabs and an Fc that is formed by a dimer of three constant domains (C.epsilon.2, C.epsilon.3, and C.epsilon.4). Compared to IgG molecules, IgE has an additional constant domain (C.epsilon.2) that replaces the IgG linker region, while the IgE C.epsilon.3 and C.epsilon.4 domains are homologous to the IgG C.gamma.2 and C.gamma.3 domains. Intact IgE and Fc fragments bind with high affinity (K.sub.D.about.10.sup.-9-10.sup.-10M) to the alpha chain of Fc.epsilon.RI and mutagenesis studies.sup.6-11 have demonstrated that C.epsilon.3 domain residues are involved in binding to the receptor, consistent with the crystallographic studies of the IgE-Fc:Fc.epsilon.RI.alpha. complex. The IgE-C.epsilon.2 domains are not thought to be important for receptor binding, since constructs of the IgE-C.epsilon.3/C.epsilon.4 domains retain high affinity binding to the receptor.

Detail Description Paragraph (87):
[0113] The IgE-Fc crystal structure reveals a novel and compact closed conformation for the Fc domains. The relative dispositions of the two C.epsilon.3 domains with respect to each other and to the C.epsilon.4 domains is substantially different from IgG-Fc or the receptor-bound IgE-Fc structures (FIG. 1). The C.epsilon.3/C.epsilon.4 angle is more acute than that found between IgG-Fc C.gamma.2/C.gamma.3 domains or for the receptor-bound IgE-Fc (FIG. 1). The free IgE-Fc is more compact, as shown by its shorter overall height (FIG. 1a, .about.7 .ANG.), with overall dimensions of 58.times.63.times.40 .ANG. as compared to 65.times.64.times.36 .ANG. for IgG-Fc (FIG. 1c) and to the dimensions of the receptor-bound IgE-Fc (FIG. 1b) (see, for example, 60/189,853). The C.epsilon.3 domains in the IgE-Fc structure also approach each other more closely, as shown by the distances between loop residues indicated in FIG. 2a. The distances between the first residue in strand A of the C.epsilon.3 or C.gamma.2 Ig domains can be readily compared, minimizing differences in distance due to loop flexibility. In IgG (FIG. 2c), this distance is .about.22 .ANG. (varies some between IgG structures), in the receptor-bound IgE-Fc (FIG. 2b) this distance is .about.23 .ANG., while in the closed IgE (FIG. 2a) this distance is .about.13 .ANG.. Overall, the receptor-bound IgE and IgG-Fc structures more closely resemble each other than the unbound IgE-Fc structure.

Detail Description Paragraph (96):
[0122] Expression and crystallization of the human IgE-Fc

Detail Description Paragraph (100):
[0126] Data from the IgE-Fc crystals was initially variably anisotropic, but improved substantially when crystals were treated with heavy atoms used for derivative screening, including platinum, mercury and other metals. Based on these onservations, crystals were treated with 1 mM copper (II) chloride prior to freezing and data collection. Although initial diffraction from native crystals was limited to .about.3.0 .ANG. resolution and often exhibited split lattices, copper-treated crystals diffracted to at least 2.3 .ANG. resolution, with little anisotropy lattice problems. This improvement may be due to the oxidation of residual free cysteines (<5%) in the IgE-Fc N-terminal residues prior to freezing. Data were collected from these crsytals at SSRL beamline 7-1 using a Mar300 imaging plate system and at the Advanced Photon Source DND-CAT 5Idbeamline, using a MarCCD detector. Native and derivative data were processed and integrated using the HKL suite of programs.

Detail Description Paragraph (129):
[0154] IgE antibodies mediate anti-parasitic immune responses and the inflammatory reactions of allergy and asthma. This Example describes the solution of a crystal structure of the human IgE-Fc C.epsilon.3-C.epsilon.4 domains to 2.3 .ANG.

resolution, the coordinates of which are disclosed in Table 2 and Table 3. The IgE-Fc crystal structure reveals a novel, closed conformation for Fc domains. For example, the structure reveals a large rearrangement of the N-terminal C.epsilon.3 domains when compared to related IgG-Fc structures and to the IgE-Fc bound to its high affinity receptor, Fc.epsilon.RI. The IgE-Fc adopts a more compact, closed configuration that places the two C.epsilon.3 domains in close proximity, decreases the size of the interdomain cavity and obscures part of the Fc.epsilon.RI-binding site. Unique structural features of the C.epsilon.3-C.epsilon.4 interdomain interfaces are identified that may enable this conformational flexibility. Fc domain flexibility may allow IgE to form optimal interactions with both of its receptors, Fc.epsilon.RI and Fc.epsilon.RII. The structure of the IgE-Fc suggests new strategies for anti-allergy treatments including the design of molecules that act allosterically to block receptor binding.

Detail Description Paragraph (138):
[0163] The IgE-Fc was purified to homogeneity and crystallized. Crystals belong to space group P42.sub.12 with cell dimensions a=b=105.6 .ANG., c=47.1 .ANG.. The crystals contain a single IgE-Fc chain (half of the dimeric molecule) in the asymmetric unit, with the molecular dimer axis lying along a crystallographic dyad. The crystals diffract X-rays to 2.0 .ANG. using synchrotron X-ray sources. Molecular replacement searches using a variety of IgG-Fc models were unsuccessful, as were heavy atom searches. The IgE-Fc C.epsilon.3-C.epsilon.4 structure was solved by an automated molecular replacement search using .about.12,000 distinct conformational variants of core models for the two Ig domains, systematically varying the angles relating the C.epsilon.3 and C.epsilon.4 domain models. Data collection and refinement statistics are shown in Table 7. The current R.sub.free and R.sub.work are 27.0% and 24.2%, respectively, to 2.3 .ANG. resolution. There is no density for the ten amino-terminal residues of the protein (including the interchain disulfide) and the four C-terminal residues. In addition, the density for the C.epsilon.4 AB loop is poor.

Detail Description Paragraph (144):
[0169] The crystal structure of the IgE-Fc C.epsilon.3-C.epsilon.4 domains reveals a novel, closed conformation for antibody effector domains (FIG. 8). In the free IgE-Fc, the C.epsilon.3-C.epsilon.4 interdomain angle is more acute than that observed between homologous IgG-Fc domains (Deisenhofer et al., 1976; Harris et al., 1999) or in the Fc.epsilon.RI-bound IgE-Fc (open conformation, Garman et al., 2000). Both the relative dispositions of the two C.epsilon.3 domains with respect to each other and to the C.epsilon.4 domains is altered. In the closed structure, the IgE-Fc C.epsilon.3 domains are closer together and slightly rotated with respect to each other. A top view of the C.epsilon.3 and C.gamma.2 domains illustrates differences in the interdomain gap (FIG. 8b). In the IgE-Fc, the distance between the first residue of the C.epsilon.3 A strands is only 13 .ANG.. The distance increases to 23 .ANG. in the receptor-bound IgE-Fc, which is similar to the 22 .ANG. observed between the C.gamma.2 domains in IgG2a-Fc (Harris et al., 1997). The C.epsilon.3 domains not only approach each other more closely, but they also lie closer to the C.epsilon.4 domains. For example, the top of the C.epsilon.3 domain (residue T396 in DE loop) is 23 .ANG. from the top of the C.epsilon.4 domain (residue S491). The distance between the corresponding residues in IgG2a is 33 .ANG., and in the receptor-bound IgE-Fc (open form), the distance is 31 .ANG.. Thus, in the change between the open and closed forms, the top of each C.epsilon.3 domain moves 10 .ANG. towards the other C.epsilon.3 domain across the dimer axis and 8 .ANG. towards the C.epsilon.4 domain of the same chain. The closer approach of the upper domains of IgE (C.epsilon.3) to the lower domains (C.epsilon.4) decreases the overall height of the IgE-Fc by .about.7 .ANG. compared to the IgG-Fc. The IgE-Fc conformational change is much greater than any differences observed among IgG-Fc crystal structures. Six crystal structures of the IgG-Fc provide nine different observations of a single chain of the IgG-Fc (in three structures, the two chains are constrained by crystallographic symmetry to be identical). These nine IgG-Fc chains, aligned via their C.gamma.3 domains, reveal IgG-Fc conformational variability as a family of C.gamma.2 positions (FIG. 9a). In the closed structure, the IgE C.epsilon.3 domain lies far outside the range of observed IgG-Fc conformations. When bound to Fc.epsilon.RI, the angle between the C.epsilon.3 and C.epsilon.4 domains increases and the C.epsilon.3 domains approach the observed positions for IgG C.gamma.2 domains. Some of the structural variation in the IgG-Fcs may be attributable to

sequence differences. While the human IgG structures share .about.95% sequence identity and the mouse structures have .about.67% identity, the largest difference in IgG C.gamma.2 positions occurs between the human and mouse structures which share .about.64% identity (Harris et al., 1999). However, the largest conformational change occurs between the open and closed forms of the IgE-Fc, which are identical in sequence, demonstrating the inherent flexibility of the IgE-Fc.

Detail Description Paragraph (150):
[0175] Carbohydrate is not required for high affinity binding to Fc.epsilon.RI, suggesting that it does not affect the conformation of the IgE-Fc significantly. IgA glycosylation is similarly not required for Fc-receptor binding (Mattu et al., 1998). In contrast, the presence of carbohydrate at a conserved N-linked attachment site in IgG (N297 in IgG1) is critical for maintaining Fc receptor-binding activities (Jefferis et al., 1998). Core glycosylation (-GlcN Ac.sub.2Man.sub.3) of IgG, produced in mammalian, yeast and insect cells, is likely sufficient for this carbohydrate function (Jefferis et al., 1998). Functional and biophysical studies of IgG indicate that the carbohydrate moiety has only a limited and local effect on the Fc structure (Jefferis et al., 1998). A comparison of glycosylated and aglycosylated IgG-Fc with a panel of monoclonal antibodies showed no detectable epitope differences, suggesting that global structural changes were not occurring (Walker et al., 1989). .sup.1H-NMR has been used to study the influence of glycosylation on the structure of IgG-Fc. Histidine resonances were monitored in glycosylated and non-glycosylated IgG-Fc (Lund et al., 1990; Matsuda et al., 1990). Of the five histidines monitored, only one near the conserved glycosylation site (H268 in the C.gamma.2 BC loop), reported any change in local environment. Histidines at the C.gamma.2-C.gamma.3 domain interface did not detect any structural differences. Based on the IgE-Fc:Fc.epsilon.RI crystal structure, the C.gamma.2 BC loop and DE loop containing the conserved glycosylation site are predicted to participate directly in Fc.gamma.R interactions (Garman et al., 2000). Local structural changes in these loops could affect receptor binding.

Detail Description Paragraph (158):
[0183] The IgE-Fc structure reveals an unprecedented conformation for antibody effector domains with implications for Fc-receptor binding and therapeutic intervention in human disease. The structure of the closed IgE-Fc suggests that the effector domains of antibody isotypes may have evolved structural characteristics that are associated with isotype-specific biological functions. Structural features that could influence the flexibility of the IgE-Fc include the location and packing of hinge residues and the specific interactions at the C.epsilon.3-C.epsilon.4 domain interface, such as the position and contacts of the C.epsilon.3 AB helix. Other factors that could potentially effect a change in conformation have been considered, such as the specific crystal-packing environment, the presence of high-mannose instead of complex carbohydrate, or the lack of the C.epsilon.2 domains. The present invention also includes the solution of a second crystal form of the IgE-Fc containing two IgE-Fc molecules in the asymmetric unit, both in the closed form. These five IgE-Fc chains all adopt a similar conformation, indicating that the closed conformation is not dictated by specific crystal-packing forces.

Detail Description Paragraph (162):
[0187] Other experimental evidence has suggested that IgE adopts a bent configuration in solution and that conformational changes may occur upon binding to Fc.epsilon.RI. The design and interpretation of these experiments could not have anticipated the specific IgE-Fc conformational change of the present invention. Binding of IgE-Fc to Fc.epsilon.RI (Keown et al., 1998) is characterized by a relatively large change in heat capacity (.DELTA.Cp.degree.=-815 cal/mol K), which could be in part be caused by IgE-Fc conformational changes. In contrast, binding of IgG-Fc to its homologous low affinity receptor, Fc.gamma.RIII, exhibits a smaller change in heat capacity (.DELTA.Cp.degree.=-360 cal/mol K). Fluorescence energy transfer experiments have shown that the average distance between the N- and C-termini of the IgE is only .about.70 .ANG., a distance that is possible only if the IgE bends significantly out of the plane of the typical antibody Y- or T-shape (Zheng et al., 1991). Neutron scattering studies have shown that the intact IgE-Fc (C.epsilon.2-C.epsilon.4) has a significantly more compact shape than a linear arrangement of the domains would allow (Beavil et al., 1995), suggesting that a bend occurs within the IgE-Fc region. The IgE-Fc crystal structure supports the

interpretation of bending of the intact IgE at the C.epsilon.2-C.epsilon.3 linker region, and may provide a better model for the analysis of the neutron scattering data. Experimental tests of IgE flexibility can now be developed based on the structure.

☐ 4. Document ID: US 4758511 A

L1: Entry 4 of 5 File: USPT Jul 19, 1988

US-PAT-NO: 4758511
DOCUMENT-IDENTIFIER: US 4758511 A

TITLE: CDNA clones coding for polypeptides exhibiting IGE binding factor activity

DATE-ISSUED: July 19, 1988

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Martens; Christine L.	Menlo Park	CA		
Moore; Kevin W.	San Bruno	CA		
Ishizaka; Kimishige	Towson	MD		
Huff; Thomas F.	Baltimore	MD		

US-CL-CURRENT: 435/69.5; 435/320.1, 435/466, 536/23.53, 930/10, 930/260

ABSTRACT:

Plasmid vectors are provided that carry complementary DNA (cDNA) clones coding for polypeptides exhibiting mammalian IgE binding factor activity. One of these clones contains an open reading frame consisting of 556 codons. The cDNA is derived from messenger RNA isolated from a rat/mouse T-cell hybridoma line. The cDNA was cloned by incorporation into a pcD plasmid vector. The plasmid vector also contains DNA segments from the SV40 virus, permitting expression of the cDNA to form a polypeptide having IgE potentiating activity after transfection into a mammalian host cell, such as monkey Cos7 cells.

16 Claims, 4 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 8

L1: Entry 4 of 5 File: USPT Jul 19, 1988

DOCUMENT-IDENTIFIER: US 4758511 A
TITLE: CDNA clones coding for polypeptides exhibiting IGE binding factor activity

Detailed Description Text (54):
(b) Fixed erythrocytes sensitized with IgE (IgE-RBC) or human serum albumin (HSA) (HSA-RBC): IgE was prepared from ascites fluids of rats or mice bearing rat IgE myeloma (IR183 or IR162; Baxin, H., et al., Immunology 26: 713 [1974] or mouse IgE hybridoma (ATCC Accession Nos. TIB141 and TIB142) tumors, respectively. The techniques of ammonium sulfate precipitation, DEAE-cellulose chromatography, and gel filtration are standard in the field (Mishell, B. and Shiigi, S. [eds.] "Selected Methods in Cellular Immunology", W. H. Freeman and Co. San Francisco Calif. [1980], pp. 278-280; Vander-Mallie et al. J. Immunol. 128: 2306-2312 [1982]). Since rodent IgE is acid-labile, care was taken to maintain pH>7.0 during these procedures. Fixed ox erythrocytes were washed once in 0.1M Na acetate, pH 5.0, and resuspended to 4%

in this buffer. To 0.25 ml of 1 mg/ml rat or mouse IgE or HSA (2X crystallized, Nutritional Biochemicals) in borate buffered saline, pH 8.0 (PBS), was added 0.25 ml 0.1M Na acetate, pH 5.0, followed by 0.5 ml of the fixed erythrocyte suspension. This mixture was incubated 2 hr at room temperature on a rotator as described above. The sensitized cells were washed three times with 1 ml PBS and resuspended in 1 ml PBS (2%). This suspension could be stored on week at 4.degree. C.

5. Document ID: US 4634599 A

L1: Entry 5 of 5 File: USPT Jan 6, 1987

US-PAT-NO: 4634599
DOCUMENT-IDENTIFIER: US 4634599 A

TITLE: Method for making ordered monolayers of macromolecules

DATE-ISSUED: January 6, 1987

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Uzgiris; Egidijus E.	Schenectady	NY		

US-CL-CURRENT: 427/2.13; 378/73, 428/408, 428/420, 428/478.2, 436/518, 530/387.1

ABSTRACT:

A method is provided for making ordered monolayers of macromolecules. A supported lipid polylayer is contacted with macromolecules in an aqueous polar solution and allowed to incubate.

6 Claims, 2 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 1

L1: Entry 5 of 5 File: USPT Jan 6, 1987

DOCUMENT-IDENTIFIER: US 4634599 A
TITLE: Method for making ordered monolayers of macromolecules

Detailed Description Text (7):
A similar procedure was followed by floating a silver grid having a lipid bilayer onto a solution of IgE. Incubation in the IgE (mouse monoclonal anti-DNP) was allowed to proceed for 6 hours. The composite was removed and stained with 1% uranyl acetate. It was found that substantially similar 2-D crystallization had occurred utilizing IgE. A fault-free, sheet like coverage of molecular clusters was observed everywhere. The clusters were organized into hexagonal arrays of similar spacing to the IgG arrays, but of less well defined local order as judged by optical diffraction. It packed into a 2-D lattice differently from the IgG antibody. The appearance of the IgE lattice was quite different; it was diffuse with few sharp edges. However, the extent of the domains was several microns, and approximately the same as IgG.

Detailed Description Text (12):
The degree of 2-D high density binding of IgG and IgE directly measures the fraction of the lipid layer still on the grid; it indicates that the haptenated lipid is still on the surface. As a result of the improved stability of phospholipid bilayer, significantly improved 2-D crystallization of IgG and IgE was achieved as compared

to the results obtained using the less stable phospholipid monolayer of Kornberg.

Generate Collection | Print

Term	Documents
IGE.DWPI,EPAB,JPAB,USPT,PGPB.	8650
IGES.DWPI,EPAB,JPAB,USPT,PGPB.	252
CRYSTAL$	0
CRYSTAL.DWPI,EPAB,JPAB,USPT,PGPB.	668479
CRYSTALA.DWPI,EPAB,JPAB,USPT,PGPB.	6
CRYSTALAB.DWPI,EPAB,JPAB,USPT,PGPB.	5
CRYSTALAB-INC.DWPI,EPAB,JPAB,USPT,PGPB.	1
CRYSTALAC.DWPI,EPAB,JPAB,USPT,PGPB.	2
CRYSTALAGRAPHIC.DWPI,EPAB,JPAB,USPT,PGPB.	1
CRYSTALAID.DWPI,EPAB,JPAB,USPT,PGPB.	1
CRYSTALAIRE.DWPI,EPAB,JPAB,USPT,PGPB.	1
(CRYSTAL$ NEAR3 IGE).USPT,PGPB,JPAB,EPAB,DWPI.	5

There are more results than shown above. Click here to view the entire set.

Display Format: Change Format

Previous Page Next Page